

Claims

What is claimed is:

- 5 1. An isolated polypeptide having carboxypeptidase activity, selected from the group consisting of:
- (a) a polypeptide comprising an amino acid sequence which has at least 50% identity with the amino acid sequence of SEQ ID NO:2;
 - (b) a polypeptide which is encoded by a nucleic acid sequence which hybridizes under
10 medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) its complementary strand, or (iii) a subsequence thereof;
 - (c) a polypeptide having (i) optimal activity in the range of about pH 3.0 to about pH 7.5 at 25°C; (ii) optimal activity in the range of about 55°C to about 60°C at pH 4; (iii) a residual activity of at least about 65.5% after 30 minutes at pH 4.0 and 60°C; and (iv) a
15 capability to hydrolyze X from N-CBZ-Ala-X wherein N-CBZ is N-carbobenzoxo and X is any amino acid;
 - (d) an allelic variant of (a) or (b); and
 - (e) a fragment of (a), (b), or (d), wherein the fragment retains carboxypeptidase activity.
- 20
2. The polypeptide of claim 1, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) its complementary strand, or (iii) a subsequence thereof.
- 25 3. The polypeptide of claim 2, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) its complementary strand, or (iii) a subsequence thereof.
4. The polypeptide of claim 3, which is obtained from a strain of *Aspergillus* or a
30 synonym or teleomorph thereof.

5. The polypeptide of claim 4, which is obtained from a strain of *Aspergillus oryzae* or a synonym or teleomorph thereof.
6. The polypeptide of claim 1, which has (i) optimal activity in the range of about pH 3.0 to about pH 7.5 at 25°C; (ii) optimal activity in the range of about 55°C to about 60°C at pH 4; (iii) a residual activity of at least about 65.5% after 30 minutes at pH 4.0 and 60°C; and (iv) a capability to hydrolyze X from N-CBZ-Ala-X wherein N-CBZ is N-carbobenzoxo and X is any amino acid.
7. The polypeptide of claim 6, which hydrolyzes N-CBZ-Ala-X wherein X is selected from the group consisting of Ile, Glu, Lys, Arg, Asp, Asn, Phe, and Tyr.
8. The polypeptide of claim 6, which is obtained from a strain of *Aspergillus* or a synonym or teleomorph thereof.
9. The polypeptide of claim 8, which is obtained from *Aspergillus oryzae* or a synonym or teleomorph thereof.
10. The polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:2 or an allelic variant or fragment thereof which retains carboxypeptidase activity.
11. The polypeptide of claim 10, comprising the amino acid sequence of SEQ ID NO:2.
12. The polypeptide of claim 11, consisting of the amino acid sequence of SEQ ID NO:2.
13. A polypeptide having carboxypeptidase activity encoded by the nucleic acid sequence contained in plasmid pEJG12 contained in *E. coli*, NRRL B-21616.
14. An isolated nucleic acid sequence encoding the polypeptide of claim 1.

15. A nucleic acid construct comprising the nucleic acid sequence of claim 14 operably linked to one or more control sequences which direct the expression of the polypeptide in a suitable expression host.
- 5 16. A recombinant expression vector comprising the nucleic acid construct of claim 15, a promoter, and transcriptional and translational stop signals.
17. A recombinant host cell comprising the nucleic acid construct of claim 15.
- 10 18. A method for producing the polypeptide of claim 1 comprising (a) cultivating a strain, which in its wild-type form produces the polypeptide, to produce a supernatant comprising the polypeptide; and (b) recovering the polypeptide.
19. A method for producing the polypeptide of claim 1 comprising (a) cultivating a host
15 cell comprising a nucleic acid construct comprising a nucleic acid sequence encoding the polypeptide under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.
20. A method for producing a mutant of a cell, which comprises disrupting or deleting
20 a nucleic acid sequence encoding the polypeptide of claim 1 or a control sequence thereof, which results in the mutant producing less of the polypeptide than the cell.
21. The mutant produced by the method of claim 20.
- 25 22. A method for producing a heterologous polypeptide comprising (a) culturing the mutant of claim 21 under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.
23. A method of producing a hydrolysate from a proteinaceous substrate which comprises
30 subjecting the substrate to a polypeptide of claim 1 and an endopeptidase.
24. A protein hydrolysate produced by the method of claim 23.

25. A food product comprising the protein hydrolysate of claim 24.
26. A method of obtaining from a proteinaceous substrate a hydrolysate enriched in free glutamic acid and/or peptide bound glutamic acid residues, comprising subjecting the
5 substrate to a deamidation process and a polypeptide of claim 1.
27. The method of claim 26, further comprising subjecting the substrate to one or more unspecific acting endo- and/or exo-peptidase enzymes.
- 10 28. A protein hydrolysate obtained by the method of claim 27.
29. A food product comprising the hydrolysate of claim 28.

Handwritten signature/initials